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SELECTED EDITORIAL

THE WELLCOME TRUST*

FOREWORD: This Editorial, reprinted here, from the British Medical Journal, describes an unusual form of legacy dedicated to a cosmopolitrn philanthropy. Sir Henry Wellcome, a graduate of the Philadelphia College of Pharmacy, has willed to the whole world, the proceeds] of his world-wide well-ordered business; ever so long as that business shall last. It is sincerely hoped that the trustees will find it expedient and proper to allot no inconsiderable share of the money, when available, to further research in Pharmacy, the field where Sir Henry's grain was grown and garnered.

MANY years ago a book was published in America under the title *Inspired Millionaires.* Instead of denouncing those who had accumulated enormous wealth an endeavour, said the author, should be made to inspire them. The process of inspiration has certainly had some success, especially in the country of Rockefeller and Carnegie. The British public has scarcely recovered its breath after the Nuffield benefaction to Oxford before another great philanthropic endowment, of a different character but directed to similar ends, is announced in the shape of the Wellcome bequests. A letter from the five trustees appointed under the will of Sir Henry Wellcome, who died in July last, explains that after some personal provisions have been made, including a memorial in Minnesota to the testator's parents, the whole of the divisible profits in Wellcome Foundation Limited will be utilized in two categories—namely, a "Research Undertaking Charity" for the advancement of medical and scientific research, and a "Museum and Library Charity" for the establishment or endowment of research museums and libraries. The Wellcome Foundation was established in 1924 by Sir Henry Wellcome to take over the business of Burroughs Wellcome and Company, manufacturing chemists, with their subsidiary companies in other parts of the world, together with the Wellcome Research Institution, which in-

*From the *British Medical Journal.*

cludes the physiological and chemical research laboratories and the museums of medical history and medical science in London bearing Wellcome's name, as well as laboratories elsewhere. Although for convenience Sir Henry Wellcome registered the Foundation as a limited company he was its sole possessor.

Unlike most other great legacies, the Wellcome gift cannot be estimated in actual figures. A statement appeared in the Press that it represented the income from one million pounds of industrialized capital, or probably substantially more—a figure no doubt suggested by the fact that the company was registered with a capital of one million one pound shares. No information is really available, outside the directorate of the Foundation, on which an estimate can be based. The point is, however, that here we have dedicated to research, not a fixed sum in trustee securities, but the profits and good-will of one of the best-known businesses in the country, enjoying a high reputation, with interests more or less all over the world. It will be run in future, not for private nor even for co-operative profit, but for the endowment of medical and allied scientific research and the upkeep of scientific institutions. Bequests, even on the grand scale, are not infrequent, but businesses dedicated in perpetuity are rare. One instance of which we have been reminded is to be seen at Copenhagen in the Carlsberg Foundation. Here the founder and proprietor of a brewery originally started research laboratories for the benefit of his business, and at his death left a large part of the earnings of his very successful brewery to trustees with such wide discretion that the Foundation has been a fairy godmother to sciences and arts having little connexion with the actual business of brewing. In the Wellcome bequest, however, the machinery whereby the revenues are earned is in character with the purposes to which they will be devoted, although the purposes, of course, spread out over a much wider field. It may be reasonably pointed out that commercial undertakings, however well established, have usually a short life as compared, for example, with ecclesiastical and university institutions; but those who in future turn expectantly to the largesse which the Wellcome trustees may be able to distribute will not be depressed—and will have no reason to be—by doleful predictions based on the insecurity of commercial and industrial affairs.

By the terms of the will the Foundation remains under a board of directors, the managing director at the time of Sir Henry Well-

come's death, Mr. George E. Pearson, becoming governing director. The function of the five trustees appointed is not to concern themselves with the detailed conduct of the business, which calls for expert direction, but to act as the equivalent of a body of shareholders, holding the property on behalf of those whom the testator intended to benefit and distributing the dividends according to his directions. The will directs that two of the trustees shall always be men of standing and authority in medical and allied scientific research. No more fitting choice could have been made than Sir Henry Dale, director of the National Institute of Medical Research, who for ten years, from 1904 to 1914, was associated with Sir Henry Wellcome as director of the Wellcome Physiological Research Laboratories, and Professor T. R. Elliott (in place of the late Sir Walter Fletcher, who was first nominated) of University College Hospital. Sir Henry Wellcome was concerned for the soundness of the research which his gift would make possible. There is an italicized statement in the will, that "persons of rash and speculative tendencies should be barred" (as trustees). Other parts of the testamentary phraseology bear a certain likeness to the language employed by the Medical Research Council in the pamphlet describing its own constitution and functions. The medical trustees whom the will named were persons who, in one capacity or another, were concerned in the work of that body, and therefore likely to be in sympathy with the testator's ambitious projects. At the time the will was made (February, 1932) Sir Walter Fletcher was secretary of the Medical Research Council; Sir Henry Dale has long been closely associated with its work, and Professor Elliott was formerly a member and known to Sir Henry Wellcome in that capacity. Of the other trustees two are solicitors and one a chartered accountant. The number of trustees will always be five, and express provision is made in the will that two shall be men of medical eminence, two shall be men of business and administrative experience, at least one of them of high standing and ability in the law, and that all shall be persons in sympathy with the work of the Foundation.

The trustees are given very wide powers. While no doubt one of their cares will be to provide for researches to be carried on in the existing Wellcome institutions, they are by no means limited to these. They can use money for the support of research work in a much larger field of inquiry, no matter where it may be carried on, and without

reference to nationality or race. They can subsidize at their discretion virtually any form of medical research. Their aim, to quote the words of the will, is to be "the advancement of research work bearing upon medicine, surgery, chemistry, physiology, bacteriology, therapeutics, materia medica, *pharmacy* and allied subjects, and any subject or subjects which have or at any time may develop an importance from the invention and improvements of medicinal agents and methods for the prevention and cure of diseases and control or extermination of insect or other pests." Grants can be made either to individuals or to institutions. Any form of research can be subsidized independently of the laboratory in which it is undertaken. Commissions of investigation can be set up. Provision may be made for the publication of the results of research. Special research expeditions—one of Sir Henry Wellcome's particular interests—may be fitted out. The furnishing and equipment of museums, libraries, research bureaux, and laboratories may be provided. Perhaps it is well to add—to prevent the trustees from being overwhelmed by appeals for all manner of researches, deserving or not—that for the next year or two there will be unknown demands on the realized profits of the Foundation. There may well be supplemental sums to be found for death duties, and there will have to be preferential provision for the personal bequests named in the will. But eventually there can be no reasonable doubt that a very large sum will be available annually for the widest purposes of research which is likely to conduce to the improvement of the physical condition of mankind.

Attention is drawn to a proviso on which the medical trustees have insisted. A clause in the will calls upon the trustees to enter into a covenant not to be engaged in or in any way to assist any concern carrying on business in competition with the Wellcome Foundation. A form of words has been added to the covenant, by order of the High Court, whereby any implication incompatible with the position of the trustees as members of the medical profession and independent men of science has been removed, the other three trustees, who were not affected, co-operating in these proceedings. The order not only meets the case of the present two medical and scientific trustees, but empowers any such trustees appointed in future to add this proviso to their covenant. In taking this action Sir Henry Dale and Professor Elliott have evidently intended to make it plain that their acceptance of trusteeship is on conditions which place its scientific independence

beyond question. The trust has every appearance of being a big undertaking,perhaps bigger in the next generation than it can be in this. Upon the trustees, especially those representing medicine and the ancillary sciences, a great responsibility will rest for the worthy investment of the sums which periodically become available. Even the trustees themselves cannot as yet see clearly "the shape of things to come." The acceptance of the trust could only be regarded as a public duty—a duty not only to the past, to carry out the wishes of a testator of philanthropic vision, but also to the future, to the various work with unimagined possibilities which may be started as a result of this inheritance. In undertaking the duty, which will not be an easy one, the medical trustees will enjoy the confidence of all their scientific colleagues, and may add another noteworthy chapter to their own eminent services to medicine.

"There is nothing so practical in its values as accurate knowledge, and the pursuit of such knowledge has been most successful when not fettered with the initial demand that it be directed toward practical ends."

—K. T. Compton in Address as Retiring President of the American Association for the Advancement of Science.

ORIGINAL ARTICLES

MECHANISM OF STRYCHNINE.

I. THE PHYSIOLOGICAL EVALUATION[1]

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I. INTRODUCTION

Pioneering for ten years with a new living reagent—daphnia—by the senior author and his students, or associates, has made it possible to solve the intricate problems of great scientific, medicinal and economic importance, discussed herein.

STRYCHNINE, an arrow poison, well known for over one hundred years, has been used extensively in the practice of medicine as a general stimulant. Of even greater economic importance has been its use as a pest exterminator in both home and field.

The importance of strychnine from a therapeutic and toxicological point of view has been emphasized by medical men and pharmacologists (3, 9, 14, 18, 19). Burn (1) refers to its as being commonly considered as a tonic and outstanding stimulant. In Merck's Manual of Therapeutics and Materia Medica, (10) strychnine is recommended as a general antidotal stimulant.

Caillé (2) reports that "in the mind of close observers, strychnine has been a disappointment as a stimulant." We feel, as some other workers do, that its physiological importance has been overrated. The final judgment of the medicinal value of strychnine depends upon the complete understanding of the mechanism of its action. This in turn depends in some degree on the purity and concentration as determined by adequate methods, chemical and physical and, if necessary, physiological.

No doubt exists as to the deadly effect of strychnine as evidenced by its use in ton quantities for the eradication of pests, such as mice, rats and other rodents, wherever they aggregate. But here again,

*Presented at Amer. Assoc. Advancement of Science, Denver, June, 1937.

[1] Contribution from the Biological and Biochemical Research Laboratory, supported by Mr. William H. Gross.

workers disagree as to how to interpret the apparent lack of its uniformly physiological efficiency in this respect. Most workers regarded the failure of certain doses to kill as the result of the varying resistance of individual animals, as well as of groups of animals, to the usually fatal doses of strychnine. Coincident with this concept of animal variability went the general satisfaction that since strychnine is a crystalline substance, its purity, uniformity and general toxic efficiency could be assured by the recognized methods of chemical and physical standardization.

The well-established basis for this concept has been undermined by a series of recent contributions from workers connected with the U. S. Biological Survey, (12, 26, 27). In lectures and publications, evidence has been presented of marked variations, well over 300 per cent., when strychnine was administered orally to rats, and 200 per cent. when it was injected. The strychnine was of high commercial purity as attested by chemical and physical analyses, showing ±99 per cent. of pure alkaloid base. The apparent variations in lethal potency were interpreted by the workers as evidence of an inadmissible deficiency of certain designated lots. These, they judged to be either partially undesirable or altogether unsuitable for pest eradication. Were such diversity existent for pure strychnine, it might conceivably occur also in the administration of other pure therapeutic and toxicological substances.

In order to accomplish the physiological evaluation and establish its significance, a detailed investigation was made in which we used both invertebrates and vertebrates. Prior to discussing our methods and results, we desire to emphasize that every effort was taken to standardize our test animals. Recognizing that death is too variable an end phase in any quantitative physiological evaluation (with time as an integral factor), we undertook to solve the problem by focussing our attention on degrees of such specific debilities, which permitted a physiological comparison of the action of pure strychnine obtained from varied commercial sources.

II. HISTORICAL

The toxic symptoms produced by strychnine in animals have often been described. Quantitative experiments have shown: (1) a relation of dosage to varied toxic effects such as hyperexcitability,

convulsion and death; (2) a varying resistance of the same type as well as of different types of animals to strychnine.

Munch (11) records the following results of some workers, which are in part presented here:

TABLE I

PHYSIOLOGICAL ACTION OF STRYCHNINE ON ANIMALS

Milligrams per Kilogram

	Oral Administration					Hypodermic Administration						
Symptoms	Cat	Dog	Guinea Pig	Rabbit	Rat	Cat	Dog	Frog	Guinea Pig	Mouse	Rabbit	Rat
Hyperexcitability	0.1	0.075	40.0	—	—	0.08	0.075	—	3.0	—	0.2	—
Convulsions	—	0.175	—	—	—	—	0.1	0.5	—	0.6	0.3	—
Death	1.0	0.25	44.0	4.25	25.0	0.32	0.3	5.5	3.0	0.75	0.5	3.0

Also recorded are the reports: (1) of increased susceptibility to strychnine (a) of some species over others (e. g., the water frog over the grass frog); (b) upon increase in temperature; (2) of observations that the injection of over-doses into frogs (several milligrams) did not produce tetanus but did cause paralysis of several days duration.

Frogs

Focke (5) suggested that the quantitative determination of strychnine might be possible by ascertaining the time when convulsions occurred after injection of various amounts of strychnine into frogs. Various other authors, however, considered frogs unsuitable for the standardization of strychnine. Recent workers have emphasized the need for uniform feeding or starvation to assure more consistent results (28).

Cats

According to Hatcher and Eggleston (7), cats were found to be more convenient than frogs, and bioassays gave more accurate and convenient quantitative estimations of strychnine in tissues than chemical assays.

Bitter Taste

Differences of but 10 per cent. in the concentration of strychnine, as determined by the perception of the bitter taste, could, after standardization of the taste, be very readily detected by Munch (11) and 5 per cent. "without trouble" by Ward and Munch (25). Munch (11) concludes: Tests upon mice, upon frogs by lymph-sac and intraspinal injections, and determinations of the threshold of bitter taste serve for the quantitative determination of strychnine.

None of these methods, however, have been adopted in the later work carried out by Munch and co-workers of the U. S. Biological Survey. In their investigation rats were used and the strychnine was orally introduced into starved animals. The results were, at first, based upon death and, later, supplemented by the onset of convulsions as experimental criteria. The great variability in the number of rats dying after strychnine was orally administered has already been emphasized by Schwartze (15) as being related to the net quantity of strychnine effective in the blood stream after absorption. This explanation might conceivably be used to interpret as well similar variations observed by numerous investigators for animals other than rats and belonging to both invertebrates and vertebrates. (See Table II.)

III. CHEMICAL AND BIOLOGICAL REAGENTS AND METHODS

Chemicals

Through the cooperation of Mr. Joseph Rosin, Chief Chemist and Vice-President of Merck & Company, eight samples of commercial strychnine base of varied origin were obtained for testing. These were designated by lot number only and the manufacturers' identity remained unknown to us.

9123	48127	47914	51528
9047	46644	48483	52415

The samples behaved alike when dissolved, in that persistent warming was necessary in order to effect true solution. Some chemical and physical tests were made on the submitted samples of commercial strychnine. No marked differences in their reactions were observable. All samples were free from brucine, according to U. S. P. tests, and the studies of the crystals with polarized light were in agree-

TABLE II

TOXIC AND LETHAL DOSES FOR STRYCHNINE

Resistance to Strychnine Base and Salts

Animal	Administration Oral	Lethal dose	Subcutan.	Lethal doses	Intraven.	Lethal doses	Remarks	Literature General
Frog (50g)	20 mg.	per frog	2-10 mg.					Heffter II (8) p. 387
			0.035 mg.	per frog			1/1400 mg. tetanus without paralysis after 1 hr.	Fleury & Zernick (4) Igersheimer (4)
			0.35 mg.	per kg. b.w.			1/140 mg. tetanus with paralysis after 10 min.	
			2.1 mg.	per kg. b.w.				Falck (8)
Water Turtle	100 mg.	per turtle					death without preceding cramps	Tierchemie 38, 1131, 1909
White Fish			6.2-12.5 mg.	per kg. b.w.				

TABLE II (Continued)

Animal	Administration Oral	Lethal dose	Subcutan.	Lethal doses	Intraven.	Lethal doses	Remarks	Literature General
Cat	0.75 mg.		0.75 mg.	per kg. b.w.	0.3-0.35 mg.	per kg. b.w.		Falck (4) Hatcher (11) Smith
Cattle			0.3-0.4 g.	per cow or ox				Froehner (4)
Chickens	30-140 mg.	per kg. b.w.	3-5 mg.	per kg. b.w.			Slow resorption	Froehner (4)
Choloepus (Hoffmanni): Sloth	0.6 mg.	?					death after 8 days	
Dog	0.75 mg. 1.12 mg.	per kg. b.w.	0.5 mg.	per kg. b.w.				Rieder Froehner (4) Falck

TABLE II (Continued)

Animal	Administration Oral	Lethal dose	Subcutan.	Lethal doses	Intraven.	Lethal doses	Remarks	Literature General
Dog	0.3-0.4 mg. (sometimes)	per kg. b.w.	0.3-0.4 mg. often fatal after 12-75 min.	per kg. b.w.	0.2-0.3 mg.	per kg. b.w.	orally 0.3-04 mg. 1) no effect 2) toxic effct 3) fatal effect	Froehner (4) Schäklee (4) Meltzer (4)
Ducks	3-4.5 mg.	per kg. b.w.	1-1.1 mg.	per kg. b.w.				Froehner (4)
Goose	25 mg.	per kg. b.w.	1-2 mg.	per kg. b.w.				Froehner (4)
Guinea pig			3-3.4-5 mg. 3.0-3.4 mg.	per kg. b.w.			5 mg. in cold season	Falck et al.
Horse	2 mg.	per kg. b.w.	0.4 mg. 0.2-0.3 g.	per kg. b.w. per horse			Strychnine sulphate	Froehner (4)
Mice (White) 14-15 days Adult			0.5-1.25 mg. 0.02 mg. 0.03 mg.	per kg. b.w. per kg. b.w. per kg. b.w.				Falck et al. (4) Falck et al. (4) Munch et al. (12)

TABLE II (Continued)

Animal	Administration Oral	Lethal dose	Subcutan.	Lethal doses	Intraven.	Lethal doses	Remarks	Literature General
Pig		per kg. b.w.	0.6-0.7 mg.	per kg. b.w.	0.1-0.15 mg.			
Pigeons	6-11 mg.	per kg. b.w.	1-1.5 mg.	per kg. b.w.			orally in crop	Froehner (4)
Porcupine			1-6-8 mg.	per kg. b.w.				
Rabbit	1-3 mg.	per kg. b.w.	0.4-0.6-1.0 mg.	per kg. b.w.	0.2-0.5 mg.	per kg. b.w.		
Rat	7.5-25 mg. 20-25 mg.	per kg. b.w. per kg. b.w.	3.0 mg.*	per kg. b.w. per kg. b.w.	0.2-0.5 mg.	per kg. b.w.	Strychnine base used *50% mortality	Schwartze (15) Munch (11)
Sheep	4 mg.	per kg. b.w.	0.4 mg.	per kg. b.w.				Also see Ward and Garlough (24)
Child	few mg.	per child						
Man	30-40 mg.	per man						

ment. In addition, comparisons were made under the ultra-violet light to detect, if present, any variations that might reveal themselves through fluorescence. Strychnine fluoresces but weakly as compared with brucine and between the samples no absolute differences were apparent. Slight variations of questionable significance, almost imperceptible, were recorded, only after considerable study.

Samples 48483 and 48127 were of a light bluish violet; samples 9123, 51528 and 52415 showed more violet hue, while samples 47914, 46644 and 9047 were intermediate in character when observed in irregular heaps of powder. These slight variations tended to become more obscure when the samples were carefully spread. When the samples were dissolved, and uniformly infiltrated into filter paper, no differences were observable in the marginal rings of strychnine formed on drying. The slight differences detected with the masses of powder may well be due to the varying degree of compactness.

Biological Reagent

The choice of *Daphnia magna* as the test-animal for the evaluation of commercial strychnine was made only after an extensive survey with the other animals which might possibly be used. Several hundred guppies and goldfish were studied and their responses to the action of strychnine recorded. Frogs proved to be of little value, while kittens, dogs and rats, although yielding valuable information, provided little insight as to mechanism of strychnine as compared with data gathered from studies on daphnia. The chief objections were that the above named test animals could not be sufficiently standardized and, secondly, reactions of the internal organs could not be seen or understood, unless recourse was to be taken to elaborate and expensive equipment, neither available to us nor adequately developed. The transparency of daphnia which permits the study of the internal organs with the usual optical systems and the fact that daphnia can be standardized and be had in unlimited numbers decided us in this choice. In addition, familiarity with the animal was taken into consideration (20, 22).

The propagation of daphnia for experimental use has been reported by one of us (21). In order to achieve standardization of the animals, the following cultural method was adopted. The media was made from well-aerated tap water, by adding 125 mg. soy bean flour (Cellu) and 25 mg. of urea to each gallon. A pH of 7.8-8.1 was main-

tained by adding pieces of marble (calcium carbonate). Several hundred females with embryos released into the brood sac were isolated and placed individually into a one ounce bottle, three-fourths filled with media and containing a small piece of marble.

The "expectant" mothers were checked daily at a regular time in order to separate the ones which had released their broods. The mothers were pipetted into another bottle containing fresh media, while the young were all placed in a gallon jar, which was never more than half filled in order to eliminate variations in oxygen tension. The usual piece of marble was added to prevent the media from becoming acid, thereby discouraging the growth of other organisms (Saprolegnia) inimical to Daphnia. With the temperature uniformly maintained at ± 70° F. the culture media and methods the same, the animals all genetically alike, standardization was achieved and thousands of animals of the same age were at hand for experimental use.

Material and Methods

Distilled water is toxic to daphnia, hence all test solutions were made up from culture media. The solubility of strychnine base is 1:6,600 in cold water. Concentrations of 1:20,000 were employed for the testing of the onset of convulsions and for studying mass as well as individual responses, while 1:10,000 concentration were used to hasten the characteristic toxic symptoms. The conversion of the base dissolved in media into the sulphate salt, was abandoned because of several interfering factors, principally due to buffering action of the media.

For the study of mass response, museum jars, measuring 14½ x 9½ x 1½ cm. and half-filled with the solution under test, were found to be suitable. When supported on wooden blocks containing the proper size groove, the jars could be readily handled. For the observation of the mass response in which the animals were to be individually studied, five animals were placed in as many culture bottles as needed, containing the same amount of solution.

For semi-mass response, a flat double chamber, internal dimensions of each part measuring 3 x 6.5 x 0.4 cm. was used in a specially designed stand affording four times magnification. This chamber was also used in a reconstructed lantern-slide projector, modified with an efficient cooling system so that observations could be made simul-

taneously by both of us. Using a transparent screen, rear screen projection was exclusively employed.

A micro-projector for observation at higher magnification through projection was also employed. This was specially reconstructed so that the cooling, heat and ultra-violet filtering units could operate at highest efficiency, and every precaution was taken to insure uniform operating conditions. Varying size chambers of exclusive design made from optical glass,[1] used with holders, proved to be of great value in this study. No oculars were used with the objectives of the projector. Hence, all images were primary and when viewed through the transparent screen, these images were of maximum definition combined with optimum light intensity. In addition to the above described special optical equipment, the usual compound and dissecting microscopes were used.

Special additional methods used are described in the text.

IV. DAPHNIA EXPERIMENTS

A. General Effects

Initial Toxic Symptoms

The first effects of strychnine base in dilute concentration (1:20,000) upon daphnia can be seen in the change of the style and speed of swimming. Even stroking of the antennae, usually not more than three beats and followed by a period of rest in which the animal glides through the medium or momentarily stops, is transformed into a rapid darting motion which becomes pronounced in the females by the end of two minutes. When four or more animals are followed in the smaller chamber, enlarged on the screen with the micro-projector, an apparent loss in the power of vision becomes evident. The animals collide with one another and then rest for seconds at a time. This type of behavior has been provisionally designated as sub-convulsive. It is not a stage absolutely different from mild convulsion, but only different in degree of severity. The apparent loss in vision is probably due in part to the pronounced twitching in the muscles controlling the cyclopean eye and causing sporadic rotational movement of this organ. In the meantime, corresponding twitches in the musculature of the antennæ increase in frequency and severity, and the motion of

[1] Made by Mr. A. Graham of the University of Pennsylvania Medical School.

animal is transformed into decidedly different types, easily recognizable. This is the mildly convulsive stage which in a few seconds passes over into the more violent and striking responses to the action of strychnine. The time required for these responses to become unmistakable varies from 2.4-2.75 minutes.

The onset of violent convulsions is the result of the increasing frequency of muscular twitchings in the antennæ which finally culminate into tetanus with but brief intervals of relaxation and rest. In unrestricted chambers, the tetanic beats of the antennæ force the animals to rise to the surface of the liquid, and they stay at the upper level, as a rule for hours. In the meantime, within the first ten minutes, there is a precipitate drop in the rate of heart beat and respiratory movements, and the alimentary canal is paralyzed.

Depression of Heart Beats

These results were used in some novel experiments in order to verify the report that decided differences existed between two lots of strychnine (27), which were fortunately in our hands. After the counts of the normal heart beat and respiratory movements were determined, the depressive effect of strychnine upon these organs were recorded over a definite time interval and then the animal was returned to unpoisoned culture media. When the animal was judged to be recovered, as determined by comparing its heart beat and respiratory movements with the original counts, the same animal was placed into a solution of the second sample at the same concentration and studied. No differences in their respective toxicities could be found. Additional experiments on the same animal and several other daphnia gave like results. The normal heart beat in younger daphnia is from 276-290 per minute when measured at ± 70° F., while in the older animals (20 days or more) the rate is about 236-260. There is a drop of 100 beats in the first ten minutes of strychnine action. The respiratory rate, while more variable than that of the heart, shows a corresponding precipitate drop of about 80 beats per minute.

Advanced Symptoms

As violent convulsions progress the daphnia swim upon their backs and their body is in rapid vibration. The swimming motions of looping-the-loop and somersaulting recur more frequently, and longer rest periods are the rule.

End Phase

Over a period of several hours, there is a shift from the convulsive action of strychnine to a paralytic one, which in itself is associated with the diminishing energy of the animal. Thus the animals sink lower and lower in the solution of strychnine and, finally, before lapsing into stationary convulsions on the bottom of the chamber, swim head down with an occasional feeble rise to the higher levels. From stationary convulsions to death there are several intermediate stages especially related to the debility of the internal organs, which will be described elsewhere in connection with quantitative experiments.

B. Toxicity Studies with Daphnia

Onset of Convulsions

Female daphnia of 15 days in age, without embryos in the brood sac, were used to test the toxicity of the eight samples of strychnine base dissolved in culture media at 1:20,000 concentrations. Four animals were placed by means of a suitable dropper into a specially designed chamber measuring internally 13 x 11 x 2 mm. Then the culture liquid was carefully drained away and replaced by the strychnine solution up to the half-way mark. This chamber was then placed before a similar chamber containing cool water, fixed in chamber holder, and projected immediately upon the screen. From the time the strychnine was applied to the beginning of observations, less than twenty seconds elapsed. In additional tests where more concentrated amounts of strychnine sulphate were used, it was necessary to place the chamber containing the daphnia into position on the projector before adding the solution. Using the base, each sample was tested by at least forty female animals, projected in series of four, at room temperature (70° F.).

The onset of convulsions was noted in terms of seconds for each animal by both of us, observing with stopwatches. The extremes for onset of convulsions for each sample are recorded and the average time for the onset of convulsions for all eight samples is given in the following table:

TABLE III

ONSET OF CONVULSIONS IN MINUTES

Sample	46644	52415	47914	9047	48127	48483	9123	51528
Extremes of onset of convulsions	1.5–3.5	2.05–3.45	2.13–3.33	1.58–2.91	1.75–3.43	1.33–2.66	1.8–2.75	1.33–2.75
Average onset of convulsions based on 40 animals	2.75	2.66	2.63	2.61	2.60	2.49	2.40	2.40
Deviation from mean	0.14	0.05	0.02	0.0	0.01	0.12	0.21	0.21

Total deviation = 0.76 minutes.
Average deviation = 0.095 minutes.

The agreement is quite striking, and it appears obvious that the toxicity of the 8 strychnine samples, as measured by their speed in producing convulsions under uniform conditions in *standardized* female daphnia, is approximately the same.

The time required for the onset of convulsion in male daphnia is on the average slightly less than *half* the time required for the females. Advanced stages of convulsions (tetanus) in the antennæ appear correspondingly earlier. Studied under projection, this stage is most striking, the antennæ beating ± 14 times per second. Although a smaller number of experiments were performed with males because of their scarcity, the following data appear to be of particular significance:

Males, approximately 15 days, 27 used, average onset of convulsions in all 8 samples: 1.18 minutes.

Females, 15 days, 160 used, average onset of convulsions in all 8 samples: 2.61 minutes.

The male daphnia are readily differentiated morphologically from the females in shape, presence of two copulatory hooks and of bristles on the anterior ventral portion of the shell, which in this region is inwardly curved. The presence of a prominent penis extending from

the cleansing hook is another distinguishing feature. Moreover, the male is narrower and the absence of a brood sac confers upon it a streamlined appearance as contrasted with the female. The males swim not only more erect but faster, an easily understood advantage.

Progressive Debility

In order to further determine whether any marked differences existed in the accumulative toxic effects of the eight samples of strychnine, evidenced by the inability of the animals to ascend as a result

TABLE IV

PROGRESSIVE DEBILITY AND DEATH

a=50 animals b=30 animals	Ability to ascend after 20–22 hours				Death %*
Sample	25–100%	15–25%	5–15%	Immotile to 5%	
48483 a	0	8	80	12	82
48483 b	0	3⅓	90	6⅔	100
47914 a	2	2	84	12	88
47914 b	3⅓	6⅔	86⅔	3⅓	86
52415 a	0	8	72	20	54
52415 b	6⅔	10⅔	73⅓	10	50
51528 a	4	6	66	22	66
51528 b	3⅓	3⅓	52	40	63
9047 a	4	0	62	34	38
9047 b	3⅓	3⅓	86⅔	6⅔	43
46644 a	0	2	34	64	76
46644 b	0	6⅔	66⅔	16⅔	60
48127 a	0	6	58	36	80
48127 b	0	6⅔	73⅓	20	82
9123 a	2	8	82	8	96
9123 b	0	3⅓	76⅔	20	100

*In "a" series observations made from 44-46 hours, and in "b" series from 44-45 hours.

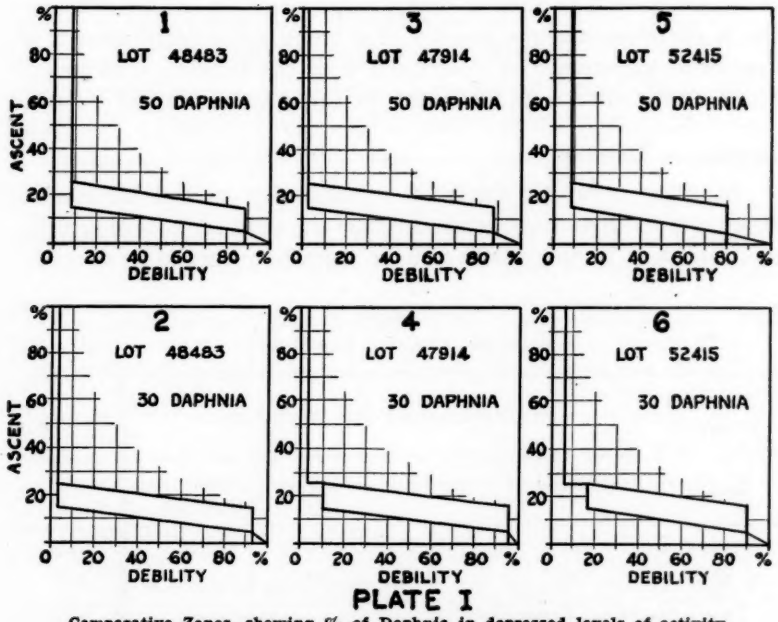

PLATE I

Comparative Zones, showing % of Daphnia in depressed levels of activity after 20-22 hours in strychnine solution (1:20,000).

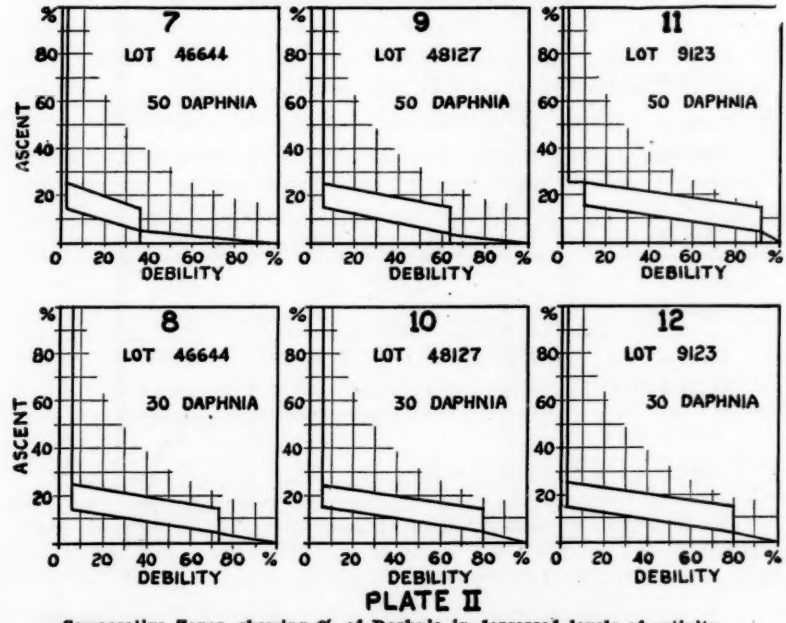

PLATE II

Comparative Zones, showing % of Daphnia in depressed levels of activity after 20-22 hours in strychnine solution (1:20,000).

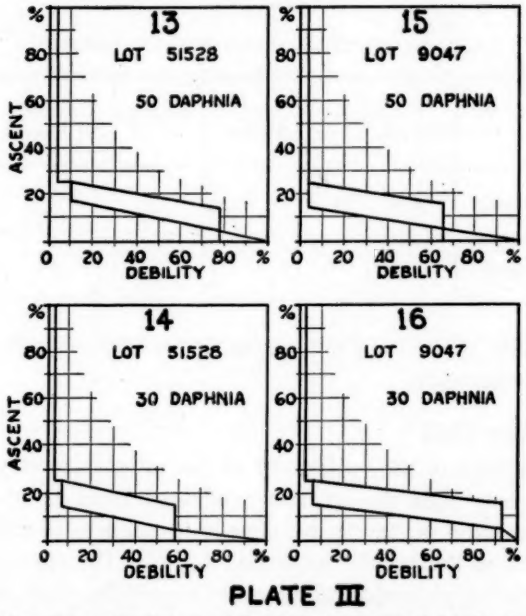

PLATE III

Comparative Zones, showing % of Daphnia in depressed levels of activity after 20-22 hours in strychnine solution (1:20,000).

of progressive debility, the following experiments were made. In one ounce culture bottles three-fourths filled with the solutions, 1 :20,000 concentration, with five daphnia to a bottle, the height to which each animal could rise at the end of twenty hours was recorded. Female daphnia, 8 days of age, without embryos released into the brood sac were used. Fifty controls, apportioned in the same way were used. The experiment was repeated under similar conditions with the exception that thirty animals per sample were employed. The temperature in both instances was ± 70° F. In order to compare the results of the two experiments with the differing number of animals, the records were converted into percentages and the action zones graphically depicted, in Plates I, II, III, from data given in Table IV.

In studying further, two samples 52145 and 48127 which according to the report were supposedly divergent (27), fifty animals were placed in museum jars half-filled with the respective samples in 1 :40,000 concentration. The animals used were all 10 day old females, without embryos released into the brood sac, and the temperature was at 70° F. ± 1°.

TABLE V

COMPARATIVE TOXICITY OF SELECTED SAMPLES

Sample 52415	20½ hrs.	22 hrs	25½ hrs.	Sample 48127	20½ hrs.	22 hrs.	25½ hrs.
No. in upper half	3–4	4	3		2–3	2	1
No. in lower tenth	46–47	46	47		47–48	48	49

Evidently no marked differences in toxicity exist at this highly diluted concentration.

Final Debility Shift

If judgment of the uniformity of the samples of strychnine had been based only upon the occurrence of death from 44-46 hours after the administration of strychnine, equally inadequate conclusions could be drawn as to the relative toxicities (*27*). Therefore, a study of the accumulative physiological effects of strychnine upon daphnia was undertaken. In order to speed up the toxic effects, 1:10,000 concentrations of the alkaloid were used. Fifty animals, 9 days old without embryos released into the brood sac, were placed in eight of the museum jars half-filled with the solutions. The temperature was ± 23° C. For the purpose of comparison, when the observations were begun at the end of twenty hours, the daphnia were classified arbitrarily into six groups:

1. Dynamic convulsions: Animal able to move for more than one body length through the convulsive action of the antennæ.
2. Stationary convulsion: Animal unable to move for more than one body length.
3. Convulsive coma: Animal unable to move, lying on side, antennæ and respiratory organs twitch convulsively, heartbeat low.
4. Coma: No twitches, otherwise the symptoms as in convulsive coma.
5. Post coma: No heartbeat, reflex action present in either antennæ, respiratory organs, or lower abdominal portion.
6. Death: No manifestation of life.

TABLE VI

FINAL DEBILITY SHIFT*

Sample	Dynam. Conv.	Sta. conv.	Conv. Coma	Coma	Post Coma	Death
51528 20 hrs.	25	10	6	3	6	1
46644 20½ hrs.	27	15	3	2	3	1
47914 21 hrs.	39	5			3	3
48483 21¼ hrs.	30	4	3	3	7	2
52415 21½ hrs.	22	13	1	5	7	2
48127 22 hrs.	26	11	3	5	2	2
9123 22¼ hrs.	15	1	0	8	3	22
9047 22¾ hrs.	16	8	1	6	8	10
51528 23½–24 hrs.	10	5	6	7	7	16

*Figures: number of animals observed.

These data are represented pictorially in their sequence in independent columns indicating the respective number of daphnia found in these progressive stages of debility and death. By joining the columns, one can readily visualize the continuity of progressive debility culminating into death. The shift to death becomes pronounced under the experimental conditions at the end of twenty-two hours.

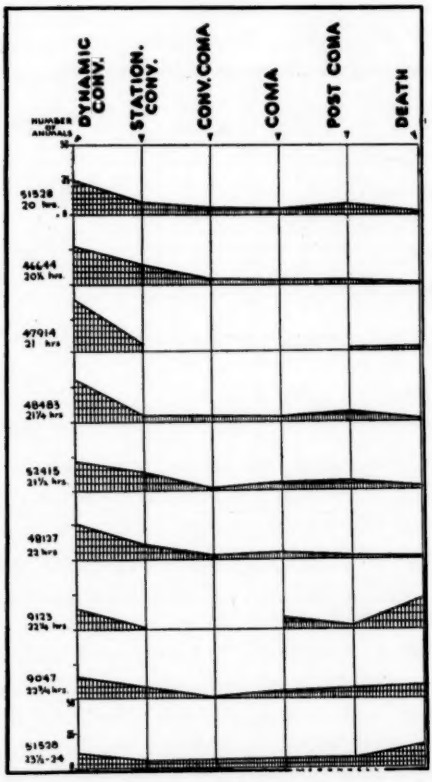

Fig. I. Comparative Shifts, depicting No. of Daphnia with specific debility symptoms after 20-24 hours in strychnine (1:10,000).

Supplementary Evidence

Detailed studies on the depressive effect of strychnine upon the heart are now in progress and will be reported in a later paper. However, we wish to include here a typical experiment that was made with these lot numbers 52415 and 48127. A large female daphnia, age 1-2 months, was placed in a suitable chamber and the heartbeat taken. Then strychnine in 1:10,000 concentration made from 52415 was administered; at the end of ten minutes the depressed rate was recorded and the animal returned to about 400 c. c. of unpoisoned culture media. After several hours, in which the depressive and convulsive effects of the strychnine had disappeared, the experiment was repeated using sample 48127 under identical conditions.

Heartbeat at start—208/min.
Heartbeat after 10 min. in 52415—116/min.
Heartbeat after washing out—196/min.
Heartbeat after 10 min. in 48127—112/min.

Similar experiments conclusively demonstrated that no differences exist between two lots of strychnine as tested by their depressive effects upon the heart of the *same animal.* Likewise, comparative studies of the depressive action of strychnine on the respiratory movements have been made. While these experiments were limited in number, the same conclusions could be drawn.

In the experiments on the effect of strychnine in 1:20,000 after 46 hours, some data as to the interference in the release of embryos into the brood sac was obtained. No deaths occurred in controls during the time of the experiment.

Daphnia with embryos released in controls: 27/80 = 33.7%.
Daphnia with embryos released in strychnine: 3/640 = 0.47 of 1%.

V. RESULTS OF CONFIRMATORY EXPERIMENTS

Palaemonetes

Another form of transparent crustacean, Palæmonetes, a fresh water prawn, when more fully studied so that it can be bred in the laboratory, may prove to be equally as valuable as daphnia. However, very delicate handling is necessary since these prawns are also very sensitive to strong intensities of light, temperature changes and varying oxygen pressure.

In Palæmonetes, after the administration of strychnine base, there is a progressive depression of the heartbeat and derangement of its regulatory mechanism, of the tetanic movement of the mouth parts and swimmerets, paralysis of the stomach and remaining portion of the alimentary canal and, finally, depression of the respiratory organs.

Flies

Contrary to the results of De Wildt (29), who found that houseflies were not poisoned by eating milk sugar mixed with strychnine, various stages of typical strychnine action were observed in flies that had fed upon milk saturated with strychnine. Hyperexcitability and convulsions consisting mainly of intermittent violent tremors of the legs were followed by paralytic coma and, finally, death.

Frog

In the frog there is at first the sensitivity to vibrational stimuli, then tetanic convulsions involving the hind legs and tetanic seizure of the mouth parts occur. Sensitivity in the frog may last for several days. The administration of sodium phenobarbital restored frogs from violent strychnine tetanus.

Guppies and Goldfish

Newly born guppies are translucent and are suitable for projection studies. However, an insufficient supply and uncertain genetical relationship from one brood to the other were serious shortcomings. The train of symptoms in guppies and goldfish was very similar and differed only in one respect in the final stages. There is, at first, an increase in the activity of the pectoral fins which causes the guppies to dart excitedly around in the container. At this stage they are very sensitive to shocks produced by rapping on the side of the container or by the footsteps of one walking into the room. The motor responses in the pectoral fins are reversed, tetanus occurs in the opercula and mouth parts and there is a loss of equilibrium. The guppies sink to the bottom of the container, while the goldfish rise to the top. This difference is due to the presence of a large swim-bladder in the goldfish. There is a gradual decrease in sensitivity to shock and finally coma sets in, where vibrational stimuli no longer affect the animals. The blood is still circulating, doing so for a few hours, and, in the meantime, is gradually withdrawn from the extremities.

Rats

In rats one of the first symptoms following a toxic dose was a slight body tremor and, upon shock, vibration of the sometimes uplifted tail, followed by rigidity. The tremors grew more severe and culminated into a convulsive spasm while the hind legs were outstretched as similarly noted in frogs, while the front legs were retracted inward towards the chest. In some instances, death followed directly, while in others, apparent recovery was followed by another violent convulsion with death then ensuing.

A single experiment on a rat (200 grams) in deep coma, induced by a feeding of 1 cc. of terpinol on the previous day in connection with other experimental work, yielded certain results which may be significant to the understanding of the mechanism of strychnine. A subcu-

taneous injection was made into the pectoral region of 0.2 cc. of 1/10 of 1 per cent. strychnine sulphate. Fifty minutes later convulsive spasms and sensitivity to shock began and continued for well over three hours. During this time, the close similarity of behavior of this rat with strychninized frogs was very striking. Terpinol, a lipoid solvent, had evidently affected the nervous system, possibly a degeneration of certain elements taking place, and the effect of strychnine given in such enormous dose (100 mg/Kg) was thereby retarded. The importance of the degree of the differentiation of the nervous system upon strychnine action will be brought out with some results obtained from dogs and kittens of varying age. (Schwartze 16.)

Dogs

Seven male puppies, all less than 10 days old, were subcutaneously injected in the lower abdominal region with 0.5 mg/Kg of strychnine base. Violent convulsions, both external and internal, were followed by labored breathing and paralysis in the hind legs. Tremors continued for several hours and recovery took place in all cases.

In contrast, a female dog, age 11 years, was given the same dosage under similar conditions. Within ten minutes violent convulsions with profuse salivation occurred and the dog fell over on her side. Labored breathing and convulsions followed alternately, until death twenty-five minutes later. The heart kept beating for 2 to 3 minutes after final relaxation. The effects of the same dosage orally on a young male dog, about one year old, were much different. There was no convulsive action, except a slight twitch in the hind legs and a temporary loss of normal locomotion. The same dog was starved until the following day and then given 1 mg/Kg orally. A violent convulsion took place followed by labored breathing. The dog was able to stand up and walk around, but had difficulty in maintaining his normal posture. Recovery followed.

Table VII summarizes certain experiments with kittens of varying age. It will be noticed particularly that the time necessary for death to occur is significantly reduced in kittens of over 4 weeks in age as compared to those of only 2 weeks. This difference may well be correlated with the degree of differentiation of the nervous system and muscular systems.

TABLE VII

COMPARATIVE TOXICITY OF SELECTED SAMPLES ON KITTENS

Litter	Sample	1st Tremor	2nd Tremor	Convulsion	Death	Notes
I 2 wks. 1. m. 320 ± 2 grams	52415 2 mg/Kg	4⅚ min.	6½ min.	8⅚ min.		All administrations oral. Kittens used without starving. Death a fading out process which requires a day. The process here does not differ markedly as observed in Daphnia. Kittens unable to sit down and locomotion not highly developed. The onset of the convulsions caused the kittens to lose their equilibrium and lie on their sides.
2. m. 320 ± 2 grams	52415 2 mg/Kg	4 min.	5 min.	8½ min.		
3. m. 320 ±	48127 2 mg/Kg	5½ min.	6¼ min.	8¼ min.		
4. m. 320 ± 2 grams	48127 2 mg/Kg	6¼ min.	7¼ min.	8½ min.		
II 4 wks. 1. m. 467 grams	52415 2 mg/Kg	5 min.		14-15 min.	24 min.	Kittens were starved for at least 24 hrs. They exhibited a different set of symptoms. When the first tremor would be felt, the kittens sat down on their haunches.
2. f. 475 grams	52415 2 mg/Kg	5 min.		14-15 min.	39 min.	
3. f. 340 grams	48127 2 mg/Kg	9 min.		15-16 min.	38 min.	
4. f. 343 grams	48127 2 mg/Kg	6 min.		14-15 min.	45 min.	

m.—Male. f.—Female.

TABLE VII (Continued)

m.—Male. f.—Female.

TABLE VII (Continued)

Litter	Sample	1st Tremor	2nd Tremor	Convulsion	Death	
III 6-8 wks. 1. f. 850 grams	52415 2 mg/Kg	6½ min.	7½ min.	8¼ min.	16 min.	Age not definitely known. Left with mother overnight. #2 kitten was the only one observed to be nursing before the experiment.
2. f. 790 grams	52415 2 mg/Kg	6½ min.		16½ min.	26 min.	
3. m. 892 grams	48127 2 mg/Kg	7 min.	12 min.	12½ min.	18 min.	
IV 10-12 wks. 1. m. 1200 grams	52415 0.91 mg/Kg	31 min.		36 min.	100 min.	Kittens unfed for at least 20 hrs. Weight of stomach 25.4 grams, weight of contents 6.8 grams.
2. m. 1233 grams	48127 0.90 mg/Kg	9 min.		18 min.	52 min.	Weight of stomach 14.6 grams empty. Entire train of symptoms different.

m.—Male. f.—Female.

The onset of tremor at the higher dosage tends to fall within a range of 4 to 9 minutes. While the onset of convulsion, though generally agreeing within each litter does not show a similar agreement between the different litters, the range of 4 kittens of 4 weeks old, and 3 kittens of 6-8 weeks old appears to fall into 12-16 minutes, with 8¼ minutes of one kitten agreeing with the 8-9 minutes range of the 2 week old kittens.

The rate of absorption alters the train of symptoms in kittens with stomach partially filled and empty. The protocols of the two kittens from litter IV shows this very clearly.

Protocol 1.

May 29, 1937.
Sample—52415.
Base—conc. 1:10,000.
Kitten—male, age definitely unknown (about 2½ months old).
Weight—1.2 Kg.
Dosage—11 cc. = 0.91 mg/Kg (Oral administration. Kitten starved for 24 hrs.).
Time administration—0.
Muscular incoordination in hind legs first noticeable after 31 minutes. Slight tremors of body.
Onset of first convulsion—36 mins.
Length of convulsion—1¼ mins.
Recovered from convulsion—40 mins.
Onset of second convulsion—95 mins.
Death—100 mins.
Weight of stomach—25.4 grams.
Weight of contents—6.8 grams.

Protocol 2.

May 29, 1937.
Sample—48127.
Base—conc. 1:10,000.
Kitten—male, same litter.
Weight—1.233 Kg.
Dosage—11 cc. = 0.90 mg/Kg (Kitten starved for 24 hrs. Oral administration).
Time administration—0.
Tremor in hind legs—9 min., 10 min.

Twitches over the body—11 min.
Hind legs paralyzed—12 min.
Extensors severely affected—13 min., 14 min.
Twitching over body—15 min.
Front legs in vigorous motion (resembling bicycle pedaling)—16 min.
Onset of first major convulsion and—18 min.
Series of intense convulsions until—52 min.
Death—52 min.
Weight of stomach—14.6 grams.
Contents—Empty.

VI. DISCUSSION

In a precise evaluation of the suspected differences in substances of a therapeutic or toxicological nature as tested upon animals, two general considerations are of paramount importance; first, chemical and physical purity of the substance, and, secondly, the uniformity of the test animals.

Ward, Munch and Garlough (26) analyzed the alkaloidal content of 27 samples of commercial strychnine from five supposedly different sources and found a variation in extremes from 98.28 per cent.-99.70 per cent., a net chemical variation of 1.42 per cent. The physiological variation of the L D 100 per cent. rats mg/Kg from over 25-7.5 mg/Kg—at least 333 per cent.—was observed when strychnine was administered orally. The two supposedly extreme lots of the base were converted into sulphate salts and solutions injected intraperitoneally in doses ranging from 10 mg/Kg to 2.75 mg/Kg. (The number of animals used is uncertain due to the incompleteness of their data.) From doses of 3-10 mg/Kg, there were no survivals, while at 2.75 mg/Kg survival was indicated for the supposedly stronger lot, while death occurred in the supposedly weaker lot.

It is difficult to understand how suspected differences in two samples would be shown when excessively large lethal doses are used. In our opinion, adequate experiments should have been performed at the critical dosage, and every essential factor evaluated in the fate of introduced strychnine as modified by physiological mechanisms of the animal. It is well known from the literature, that differences occur in various genera and orders of animals (Table II). An analysis of

this extensive material shows that a consideration of physiologcial and morphological modifications of like organ systems would tend to minimize such apparently anomalous behavior.

In birds, as an extreme sample, the presence of the crop greatly delays movements of food in the alimentary canal and one would not expect either speedy or uniform absorption. Similar explanations for morphological and physiological variations might be adduced for differences between herbivora and carnivora. Differences in respiration between aquatic and terrestrial forms, with the amphibia intermediate, might be cited.

Schwartze (15) reports evidence that substantiates his view that the accumulation of a lethal amount depended upon the rate of absorption exceeding the rate of disposal. Although the above authors (26) tried to achieve uniformity, in the selection of their experimental animals, they state certain digressions from the experimental plan in regard to numbers were necessary "owing to a lack of *sufficient uniform animals.*" [1] They offer no explanation or information as to how they evaluated the uniformity in the experimental animals used other than starving the animals for 24 hours.

While we base our conclusions mainly on results taken from daphnia, with comparatively few other animals, such as cats, rats, dogs, etc., it appears that our findings are substantiated by the experimental results on 3,000 rats carried out by Poe, Suchy and Witt (13). They report that, in preliminary tests of the toxicity of strychnine for rat, (the alkaloid being injected), a slight difference in toxicity was noted with some of their samples, but that the differences were not great because of the small lethal dose. A communication from the senior author stated that, "this difference amounted to about 10 per cent. Since the toxic dose was so small, we did not feel justified in absolutely attributing the differences entirely to the samples of strychnine." Most significant is their finding that sex and age have an important bearing on the toxicity of strychnine for rats.

In a later paper, Ward, Spencer and Garlough (27) present additional evidence purporting to the variability of commercial strychnine. Time for the onset of convulsion (T/S) and time for death (T/D) in rats, as averaged for two animals, each given oral dosages of 25, 22.5, 20, 17.5, 15, 12.5, 10, and 7.5 mg/Kg, are the basis for their evaluation. We are at a loss to understand the justification for

[1] Italics our own.

certain calculations where these workers present "interpolated averages" based on averaging rats that perished with those that survived, in order that a "proper evaluation of the alkaloid could be made" when the T/S's and T/D's of the different samples were compared. In that paper, manufacturers' lot numbers are given: B49106, B52415, B51528, B48483, B47914, B46644, B48127. They say, "In deciding about the suitability of these samples for economic poisoning use, we would promptly pass the first two lots for any purpose; we would pass the next three for rodent poisons for the less discriminating animals; and we would prefer to refuse the last two lots." The term "less discriminating animals" is left unexplained.

Schwartze (15), in evaluating his experiments on rats that had been given strychnine orally, states that, "the interpretation of experiments of this nature is met by two difficulties, first, the unreliability of death as an experimental criterion, and, second, effect of fatigue and depression, etc., upon the subsequent toxicity of strychnine." Fortunately for the clarification of this problem, obviously identical lots (6 lots of our 8 samples agreeing with 6 lots of their 7 samples) were submitted to us as we learned upon the publication of their findings (27) in March, 1937. Thus we were able to check and recheck two of the supposedly divergent lots. Our results on daphnia and kittens show no marked variation in these two lots. In interpreting the mechanism of strychnine with special reference to the degree of toxicity, certain factors must be properly evaluated and understood in the test animals. In order to compare physiological responses in test animals, their uniformity which can be achieved by standardization only, is a prerequisite. In the standardization of daphnia we have kept the importance of the following factors in mind:

1. Age: In respect to the development of the nervous, muscular, endocrine systems, and size of organs.

2. Sex: In respect to males and females; gravid and non-gravid.

3. Genetics: Homozygosity in our strain of daphnia which assures uniformity in this respect.

4. Food: Cultural technique constant throughout assuring a uniform nutritional history.

5. Temperature: Constantly maintained at 70° F. ± 1°, or at such temperature as stated.

6. Oxygen-tension: Variations eliminated by using similar containers filled to the same height with culture media, thus providing for the test animals the uniformly optimum conditions.

7. Vitality: Optimum conditions for life's activity undoubtedly assure a supply of experimental animals, where vitality is comparatively high.

The satisfactory standardization of test animals is necessary before any sound value can be attached to results dealing with the weakening or the potentiation and the synergism of strychnine (and its derivatives) obtained upon addition of other substances (6). Thus, brucine when tested with daphnia was found to be less toxic than strychnine; brucine tended to cause paralysis rather than violent convulsions.

We have found that there are no hard and fast differences between invertebrates and vertebrates when comparable organ systems are subjected to the influence of strychnine in toxic doses. No evidence suggestive that strychnine might have a stimulating effect other than hyperexcitability in both these groups was recognizable—in fact, unmistakable depression was the rule in all of our observations.

VII. SUMMARY AND CONCLUSIONS

1. The physiological evaluation of strychnine has been accomplished and its significance established.

2. Chemically and physically pure strychnine ($\pm 99\%$) of varied commercial origin, contrary to recent reports, was thus found to be essentially uniform in physiological activity.

3. This finding was made possible chiefly through the use of standardized *Daphnia magna* with visible internal organs, permitting conclusive observations and their quantitative interpretation.

4. The following specific criteria have been adopted for quantitative comparison of the eight commercial lots of strychnine tested.

Primary Evidence

a. Onset of convulsion: Occurs essentially at the same time when the eight samples of strychnine base are used in the same concentration (1:20,000) under the same experimental conditions. The time

of onset of convulsion is dependent upon the concentration of the test-solution and sex, as males showed a speedier response than females. Similar reaction times of the standardized daphnia in the samples tested under uniform conditions are used as partial evidence for physiological uniformity.

Secondary Evidence

b. The paralyzing effect of strychnine, following the initial stages of violent convulsion, can be quantitatively recorded, since the decreasing height to which the animals are restricted on their ascending motion can be measured at definite time intervals. No marked differences were observed in the effects of the eight samples tested.

c. While the earlier stages in the train of strychnine action are more reliable for quantitative determination, obvious shifts occur as the result of the accumulative toxic action. These shifts, handicapping the organ systems progressively, were found to be of supplementary significance in judging the samples uniform.

d. Death furnishes an additional basis for the evaluation of the toxicity of strychnine if time is not introduced as an integral factor in the final judgment of comparative uniformity. Death is merely a climax of progressively degenerated, physiological functions which, in themselves, permit the only insight into the severity and actual stage of debility.

e. The degrees of depression of the respiratory rate and especially that of the heartbeat of daphnia as produced by different lots of strychnine can be compared in the *same test animal.* This procedure presents a novel and valuable means of studying comparatively the effect of active substances.

5. Strychnine interferes with the normal functioning of the ovaries on Daphnia. In spite of its use in fairly high dilution (1:20,000) over a period of 2 days (46 hours), strychnine suppresses the release of embryos into the brood sac.

6. There are no sharply demarcated physiological differences between invertebrates and vertebrates with respect to comparable functional organ systems. Strychnine serves as an excellent indicator of like responses of such systems (organs of vision, circulation (heart), digestion, respiration and especially locomotion).

REFERENCES

(1) Burn, J. H.: Pharmacological Action of Tonics, *Pharmaceut. J.*, 232:113, 1934.

(2) Caillé, A.: Postgraduate Medicine, 1922.

(3) Eggleston, C.: Drugs used in the treatment of circulatory failure in acute infectious diseases, *J. A. M. A.*, 107:1213-1215, 1937.

(4) Fleury, F., and Zernick, F.: Toxische und letale Dosen für Gifte und Versuchstiere in Abderhalden, E., Handbuch der biologischen Arbeitsmethoden, Abt. IV, Angewandte Chemie u. Physik, Teil 7B, *Pharmakologie, 2*, 1935, page 1403.

(5) Focke, C.: Qualitative and quantitative determination of strychnine in criminal cases, *Vierteljahrsschr. ger. Med.* 37 (1), C. A. 1909, 3:2834.

(6) Grantham, R. I., and Munch, J. C.: Potentiation of toxicity of strychnine by quinine, *J. A. O. A. Chem.*, 14:296-297, 1931.

(7) Hatcher, R. A., and Eggleston, C.: Fate of strychnine in body, *J. Ph. Exp. Therap.* 10 (4):281-319, 1917.

(8) Heffter, A.: Handbuch der experimentellen Pharmakologie, 3d ed., Vol. 2:387, 1924, Berlin.

(9) Henderson, Y.: Respiratory stimulants and their uses, *J. A. M. A.*, 108:471-475, 1937.

(10) Merck's Manual of therapeutics and materia medica, 1934.

(11) Munch, J. C.: Bioassays, 1931.

(12) Munch, J. C., Garlough, F. E., and Ward, J. C.: Bioassays of rodenticides, *J. A. Ph. A.*, 25 (9):744-746, 1936.

(13) Poe, C. F., Suchy, J. F., and Witt, N. F.: Toxicity of strychnine for male and female rats of different ages, *J. Ph. Exp. Thera.*, 58(3):239-242, 1936.

(14) Poulsson, E.: Lehrbuch der Pharmakologie, 11th ed., 1937 (also see Heffter).

(15) Schwartze, E. W.: Observations upon the resistance of the rat to consecutive injections of strychnine, *J. Ph. Exp. Thera.*, 19:49-58, 1922.

(16) Schwartze, E. W.: Functional evidence of the phylogeny of the nervous system as shown by the behavior and resistance of the developing rat to strychnine, *J. Pharm. Exp. Thera.*, 19:273-291, 1922.

(17) Schwartze, E. W.: The Relative Toxicity of Strychnine to the Rat, U. S. Dept. of Agriculture Bull. No. 1023, 1922.

(18) Solis-Cohen, S., and Githens, T. S.: Pharmacotherapeutics, 1928.

(19) Sollman, T.: Manual of Pharmacology, 5th ed., W. B. Saunders Co., Philadelphia, 1936.

(20) Viehoever, A., and Mikuriya, A. S.: Transparent life studies, II. Effect of strychnine upon daphnia, *J. A. Ph. A.*, 21:239-249, 1932.

(21) Viehoever, A.: Daphnia propagation for experimental use, *A. J. P.*, 107:103-130, 1935.

(22) Viehoever, A.: Daphnia—the biological reagent, *J. A. Ph. A.*, 25 (12):1112-1117, 1936.

(23) Viehoever, A.: Cardinal Points in Favor of Daphnia, *Amer. Jour. of Pharmacy*, Vol. 109, pp. 260-262, May, 1937.

(24) Ward, J. C., and Garlough, F. E.: Strychnine IV. Lethal Studies on Cattle and Sheep, *J. A. Ph.* A., 25(5):422-426, 1936.

(25) Ward, J. C., and Munch, J. C.: Studies on Strychnine I. The relative sensitivity of chemical and physiological tests, *J. A. Ph. A.*, 19 (9):954-957, 1930.

(26) Ward, J. C., Munch, J. C., and Garlough, F. E.: Studies on Strychnine VI. Variation in the physiological action of c. p. strychnine, *J. A. Ph. A.*, 25 (7):590-593, 1936.

(27) Ward, J. C., Spencer, D. A., and Garlough, F. E.: Strychnine VIII. The relationship of borax and certain other chemicals to toxicity, *J. A. Ph. A.*, 26 (2):129-134, 1937.

(28) Weiss, S., and Hatcher, R. A.: Studies on Strychnine, *J. Ph. Exp. Thera.*, 19:419-482, 1922.

(29) de Wildt (see Heffter).

ABSTRACTS FROM AND REVIEWS OF THE LITERATURE OF THE SCIENCES SUPPORTING PUBLIC HEALTH

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Vitamin-C Content of Potatoes Prepared for Table Use by Various Methods of Cooking. J. E. Richardson, R. Davis, and H. L. Mayfield. *Food Research 2,* 85 (1937). As potatoes have long been recognized as a valuable source of vitamin C, it seemed desirable to determine the vitamin-C content of each type of cooked potato when it is just ready to be served. The two varieties of potatoes used were Netted Gem and Bliss Triumph, both grown in Montana. Chemical and biological tests showed the following: Approximately four grams of raw potato, from either variety, furnished a minimum protective dose for guinea pigs and the ascorbic-acid content ranged from 0.126 to 0.133 mgm. per Gm. Boiling potatoes for 35 mins. at 95° (owing to high altitude of laboratory) caused no loss of vitamin C, as measured both biologically and chemically. Quantitative chemical tests gave the following results: Steaming potatoes for 45 mins. seemed to increase the vitamin-C content; potatoes prepared in a pressure cooker at 17½ lbs. pressure for 12 mins. gave a slight loss of vitamin C; baking potatoes for one hour at 225° C. caused a definite increase in the amount of vitamin C per Gm., this, however, when corrected for loss of weight in baking showed a slight decrease in vitamin content in the case of the Netted Gem, but the Bliss Triumph evidenced a decided gain in quantity of this nutritional factor. Potatoes fried in butter (American style) retained about one-half of the vitamin-C content of the original material; potatoes fried in butter (German style) lost much less vitamin C than in the American style of preparation. Mashed potatoes, with milk, butter, and salt added, were comparable to boiled potatoes in vitamin-C potency; escalloped potatoes

retained all of the vitamin C contained in the original material, but owing to the addition of a large amount of milk, the vitamin C per gram of cooked product was diluted. The milligrams of vitamin C found in one serving (150 Gm.) of potato, cooked by these methods, have been calculated from the chemical analysis. W. G. B.

Quinine Sulfate as a Fluorescent Indicator for Precipitation Reactions. J. Grant. *The Analyst 62,* 285 (1937). A method is described whereby quinine sulfate is used as a fluorescent indicator in the titration of silver nitrate solutions with sodium chloride under ultraviolet light. At the end-point the color changes from bright pale blue to dull purple. This reaction is reversible but the color change is less sharp when titrating silver nitrate into sodium chloride. The titration may be made with 0.01 to 0.1 normal solutions, although the color change in the more dilute solutions is less sharp. The solutions must be neutral to litmus. Colored substances may be present in relatively large quantities without obscuring the end-point. This indicator has also been applied in the determination of zinc with ferrocyanide, as well as in the reverse process. The familiar bright blue fluorescence of quinine sulfate disappears with an excess of ferrocyanide ions. In this determination titrations may be carried out with solutions 0.01 to 0.1 normal. L. A. R.

Magnesium Sulfate as an Insecticide. H. W. Frings and M. S. Frings. *Science 85,* 428 (1937). V. R. Haber discovered the insecticidal properties of magnesium sulfate several years ago. His results indicated that this salt used as a spray in the proper concentration constitutes an effective control for the Mexican bean beetle. This spray has many advantages over arsenical sprays in that it is easily applied, easily removed in preparing beans for cooking and is harmless to humans if ingested. Magnesium sulfate and magnesium chloride have also been found toxic to the wheat wireworm.

Preliminary experiments on grasshopper control have shown that grasshoppers fed on a diet of bran, molasses and water, with which was incorporated 20-25 per cent. of magnesium sulfate, were as effectively killed as were those fed on a similar diet employing 5 per cent. of arsenic instead of the magnesium salt. Considerable promise

is given the use of magnesium sulfate in the control of mandibulate insects since its advantages over the arsenicals are obvious.

L. F. T.

Report on Arsenic. C. C. Cassil. *J. Assoc. Official Agr. Chem.* *20*, 171 (1937). The Gutzeit method for the determination of arsenic (*Methods of Analysis, A. O. A. C.* 1935, 370) has been subjected to some criticism. In the case of certain products that are difficult to oxidize, for example, tobacco and pine wood, it has been demonstrated that even though the arsenic is present in inorganic form certain unoxidized organic matter interferes with the evolution of arsine and causes low results. With other materials containing arsenic in organic combination, such as shrimp and cod liver oil, not only does this disturbance occur, but the arsenic is not completely converted to the inorganic form necessary for reduction to arsine. In all these cases a black color appears in the Gutzeit generator and indicates low results.

The authors investigated previously suggested methods of avoiding these difficulties. Earlier work by Gross showed that in the case of tobacco interference could be overcome by isolating the arsenic from the digested solution as magnesium ammonium arsenate and then proceeding with the Gutzeit method. Another method which has been recommended involves burning of the organic material with oxygen in an enclosed system. Both methods are long and required expert manipulation. The use of copper sulfate as a digestion catalyst frequently interferes because of subsequent precipitation of cuprous iodide and variation in rate of hydrogen evolution through activation of zinc. Mercuric oxide catalyst suffers the disadvantage of depositing mercury upon the zinc which renders the latter inert to acid. Selenium seems to be an excellent catalyst but the same difficulty is encountered as in the case of copper.

Cassil then turned to the use of perchloric acid as an aid in the destruction of interfering substances. He used this acid with several organic substances known to be resistant to the ordinary sulfuric-nitric acid digestion with the result that complete destruction of organic matter was obtained in a shorter period of time. The necessity of adding ammonium oxalate to the digested solution to destroy nitrogen oxides, which interfere with the production of arsine, is obviated

because the perchloric acid breaks up the nitrosyl-sulfuric acid, and on further boiling most of the perchloric acid is evaporated. If a small quantity of the latter remains it will not interfere in the determination.

The digestion is carried out as follows: The organic material is placed in the Kjeldahl flask and if the material is dry a little water is added to prevent spontaneous combustion. A mixture of 20 cc. of sulfuric acid and 15 cc. of nitric acid is then introduced. As soon as the initial reaction has subsided, heat is applied and more nitric acid is added in small portions from time to time as the material begins to turn brown or darken. After the second addition of nitric acid, 10 cc. of perchloric acid is added to the solution, and the heating is continued. Usually the solution clears or turns yellow (due to liberation of chlorine), but if carbonization occurs after the addition of perchloric acid a small portion of nitric acid will cause the solution to clear. Materials that contain refractory substances can be destroyed completely by prolonged boiling for 30 to 60 minutes, depending on the amount of such material present. The only absolute criterion for judging the required time for boiling is to run the Gutzeit analysis to see if the solution remains clear. Perchloric acid must not be added too soon because there is danger of an explosion if a large quantity of organic material is present. This method of sample preparation gave results which were identical with those obtained in a procedure involving ashing in a closed system.

As part of the investigation cerium nitrate and magnesium nitrate were investigated as "ash-aids" with the result that the former chemical was found to give exceedingly low results but that magnesium nitrate gave approximately 100 per cent. recovery. Cooperative work in the comparison of digestion with perchloric acid and dry-ashing is recommended.

The author also presents evidence to show that mossy granular zinc does not produce a definite rate of hydrogen evolution. Spherical mossy zinc, 20 mesh, did give satisfactory results, however.

A different method of impregnating the test strips is also recommended. The strips are placed in a 3.5 per cent. alcoholic solution of mercuric bromide and the container is then evacuated (70 mm. pressure) for a period of one hour, after which the strips are dried in

the usual manner. This procedure pulls the air out of the strips and more nearly uniform impregnation is obtained.

Finally the author recommends that other colorimetric methods be investigated to find a more sensitive and accurate method than the Gutzeit. A. O.

Chrysopsis graminifolia, Nutt.—A Preliminary Study. H. D. Rotts and H. M. Burlage. *J. A. Ph. A. 26,* 415-417 (1937). Chrysopsis graminifolia, Nutt., Compositae found in dry sandy soil from New Jersey and Delaware to Kentucky and southward to Florida, has been said to have value particularly as a poultice for sprains. The authors found the literature devoid of studies on this plant, and naturally interested because of its reputed medicinal value, investigated material obtained in the vicinity of Wiggins in Colleton County, South Carolina, collected in the Fall of 1933 just after frost, in July, 1934, and in August, 1934.

Results of their preliminary examination showed (1) moisture content varied from 9.8 to 10.8 per cent. for three lots of the entire herb collected at various seasons of the year; (2) total ash 6.26-8.33 per cent.; (3) acid-insoluble ash 0.67-4.65 per cent.; (4) presence of nitrogen, phosphorus, saponins and reducing substances, tannins and bitter principle; (5) saponins 0.36 per cent., catechol tannins 3.35 per cent., and pyrogallol tannins 0.60 per cent. M. S. D.

Potassium Chlorate in Dentifrices. From editorial *J. A. M. A. 108,* 1343 (1937). The use of potassium chlorate in mouth washes, gargles, and dentifrices, has continued over a long period of time in spite of the pharmacologic work discrediting such usage. The exploitation of pharmaceutic products containing large amounts of this salt disregards the health hazard involved. One dentifrice widely sold in the United States contains 41 per cent. of this ingredient, whereas in Austria a dentifrice containing more than 10 per cent. of potassium chlorate cannot be sold without a physician's order. Although potentially dangerous as an acute internal poison the danger of the daily use of such material as a dentifrice should also not be ignored. The Council on Dental Therapeutics will not accept any dentifrice for daily use which contains potassium chlorate.

A. P. Richardson recently (*J. Pharmacol. & Exper. Therap. 60,* 101 (1937) reinvestigated the toxic potentialities of the continued administration of chlorate for blood and tissues. He pointed out that the prolonged use of products containing potassium chlorate might result in sufficient absorption of chlorate, particularly when aided by frictional devices, local lesions, such as ulcers and pus pockets and other inflammatory states to produce injurious effects on the blood, kidneys, liver and general health. The administration of potassium chlorate has been a common practice in physiologic laboratories to produce kidney damage.

Methemoglobin formation was not found to be the first nor an important phenomenon in chlorate toxicity except just before death or postmortem. The chlorate ion and not the potassium was proven to be the toxic group.

Even though test animals survived larger amounts of potassium chlorate than is apt to be absorbed the use of chlorate in oral preparations lacks a rational or scientific basis. High concentrations, locally, may produce protoplasmic injury, as indicated by the slowing down of ciliary activity on strips of frog's esophagus.

When salt action is desired sodium chloride may be used without danger. L. F. T.

Verbena, the Holy Herb of the Romans. C. J. Zufall and B. B. Knoll. *J. A. Ph. A. 26,* 427 (1937). This is a short interesting account of the place held by Verbena in medicine, mythology, magic and superstition. Verbena was carried in times of battle by those interested in negotiations. It was a sign of protection and honor. It was a magic and an aid in prayer. It cleansed the table of Jupiter. It was a charm against disease. It was a spellmaker and it could conciliate friendship, etc. But, it must be plucked with the rising of the Dog Star and hidden from the light of sun and moon in order to be efficacious. M. S. D.

Chronic Benzene Poisoning and Vitamine C. A. Meyer. *Z. Vitaminforsch 6,* 83 (1937), through *Squibb Abstr. Bull. 10,* 553 (1937). The similarity in symptoms between scurvy and chronic benzene poisoning suggests that in the case of benzene poisoning the symptoms may be due to avitaminosis caused by the increased requirement of

vitamin C. In support of this theory a case of chronic benzene poisoning is cited wherein an abnormally high ascorbic acid requirement was exhibited. L. F. T.

Rubber Caps for Vaccine Bottles. H. Berry. *Pharm. J. 84,* 397, 431 (1937). The multiple dose container for biologicals offers considerable difficulty from the standpoint of maintaining its contents in a sterile condition following the withdrawal of one or more doses. The inclusion of a bacteriostatic agent does not entirely nor satisfactorily solve this problem. The rubber caps now used quite generally are objectionable on several counts. They either crack, yield alkaline or acid solutions on being boiled with water, are not adaptable to autoclaving when in position since they become distorted, etc.

The ideal cap should possess the following characteristics:

1. It should withstand autoclave pressure when wired on the bottle thus enabling the contents to be sterilized in the final sealed container.

2. The diaphragm should be readily pierced with a hypodermic needle without unduly blunting it.

3. It should not yield water soluble constituents, and particularly it should not alter the pH of solutions for injection.

4. The quality of the rubber should be such as to "weather" well on exposure to air and sunlight without losing elasticity and without cracking.

5. The diaphragm should not be under tension as this condition hastens weathering and the opening of cracks or punctures.

6. It should not yield solid flakes to the injection solution during autoclaving.

A "skirted" cap has been produced in a one-piece mold. This cap is made of best grade para rubber, uncolored, and made in such a manner that the center of the cap is closed by a thin rubber diaphragm 2 mm. in thickness and 9 mm. in diameter. These caps did not rupture on autoclaving although considerable "ballooning" was evidenced. After several punctures the diaphragms still withstood a pressure of 750 mm. applied in either direction.

Containers capped with these closures may be resterilized by autoclaving in an upright position after having been used several times. Although the skirted cap provides for autoclaving the sealed container and contents it does not provide protection against the carrying

through of organisms when doses are withdrawn by a syringe. Such organisms may be carried through on the hypodermic needle or may enter in the air which it is customary to inject before withdrawing fluid.

A cap has been developed by H. E. Chapman (*British Pharmaceutical Society Research Laboratory*) which protects the contents of a vaccine bottle from infection carried through by the syringe. The puncturable section of the diaphragm consists of a pad of anastomosing sponge rubber which has been impregnated with acriflavine. Air is compelled to traverse a special path part of which is occupied by this filter. The needle on passing through the cap must also pass through the sponge rubber pad. Pressure on a nipple situated above the sealing cone renders a filled container transportable without fear of leakage. Features of the cap are as follows:

1. It may be sterilized in an autoclave without ballooning or bursting and it may be reautoclaved.

2. The container may be readily filled through the cap by a hypodermic needle.

3. Successive doses can be easily withdrawn through the cap by means of a hypodermic syringe without injecting air. Air enters through the air filter and is sterilized.

4. The cap has a definite "wiping" value and would sterilize an infected needle when passed through it.

Investigations have shown claims 1-3 to be true. Feature No. 4, however, is not entirely reliable as shown by experiment. It would seem that this new cap is an ingenious attempt to produce an ideal cap and it suggests further possibilities along the line of utilizing sponge rubber as a bacteral air filter. L. F. T.

Specific Immunology Practices and Techniques. W. H. Park. *Preventive Med.* 7, 8 (1937). This is a review, giving the practical considerations in the administration of refined and concentrated diphtheria antitoxitn, diphtheria toxoid, smallpox vaccine, antiscarletinal serum, human scarlet fever immune serum (convalescent), tetanus antitoxin, tetanus toxoid, typhoid and paratyphoid vaccines, cholera vaccine, and plague vaccine. Cholera vaccine, made from spirilla heated at 58° C. for 1 hr., has proven of value in India. Results in plague immunization are uncertain.

Milk and Nutrition. I. Effect of commercial pasteurization on the nutritive value of milk, as determined by laboratory experiment (1937). National Institute for Research on Dairying, Shinfield, Reading (England), Milk Nutrition Committee report; through *Lancet 232,* 1179 (1937). The nutritional availability of calcium and phosphorus and the biological value and the digestibility of nitrogen are not affected by pasteurization. Vitamin A and carotene are not lost. There is some loss of vitamin B, probably in the B_1 fraction. About 20 per cent. of the vitamin C disappears if the milk has been exposed to light before heating. L. G.

Chemical Promises to Reduce Pneumonia Deaths to Half. Science News Letter, May 15, 1937. Successful chemical warfare pneumonia, one of the major diseases of mankind, by which there seems a good chance to reduce deaths to about half, was made public by Dr. William W. G. Maclachlan, physician-in-chief at Pittsburgh's Mercy Hospital, speaking at the opening of the Mellon Institute's new building in Pittsburgh.

The chemical used is hydroxyethylapocupreine. It is one of 76 chemical preparations based on quinine which were synthesized by a Mellon Institute laboratory staff under direction of Dr. L. H. Cretcher, and then tested on mice, rabbits, and dogs to determine whether they should be tried on human cases.

For two winters Dr. Maclachlan, and his associates have used as much of the new chemical as could be produced in treating severe cases of pneumonia. For 100 cases treated this past winter, the mortality was 27 per cent. compared with a normal mortality of about 45 per cent. for 100 non-specifically treated cases in Pittsburgh hospitals.

With due scientific caution, Dr. Maclachlan said: "We can safely say that in hydroxyethylapocupreine we have developed a quinine derivative which is devoid of any visual disturbance and which appears to have a power in affecting a certain number of pneumonia cases in man. Its exact clinical status will have to wait until a larger number of cases have been studied by others and by us."

The administration of the new chemical is extremely simple. The patient simply swallows it in capsules as though it were quinine. Gigantic doses are given, 400 to 800 grains or even higher during a week's time. The usual daily dose for adults is 120 grains.

The chemical is also effective on the kinds of pneumonias for which there is no serum, such as type three.

The beginnings of this new attack on the pneumococcus and the disease it produces go back to a German observation in 1911 that the quinine derivative, ethylhydrocupreine or optochin, had strong power to destroy the germ. But when it was tried on patients it produced temporary blindness in some cases.

In Germany, Japan and at the Mellon Institute research was pushed to produce a similar effective chemical but without the blindness hazard. Some 20,000 white mice were used in Mellon Institute experiments over the last four years until the present chemical was developed.

The chemists have succeeded in increasing the amount of the chemical produced and there is now prospect that supplies will be available for issue to other hospitals for widespread clinical trials.

"This chemical is compatible so far as we know with the use of any form of serum which may be given in certain types of pneumonia," said Dr. Maclachlan, "and further the ease of administration will make it very available for the general practitioner of medicine to use early in pneumococcic disease of the lung."

Hydroxyethylapocupreine is also useful in treating empyema, a common complication of severe pneumonia infections. A weak solution of it injected into the pleural cavity speeds healing, experience showed. Dr. Maclachlan warned, however, that it has no beneficial action on influenza or streptococcus infection.

One striking observation in the clinical tests on pneumonia was that better results were obtained when the treatment with the chemical began on the third day of the attack, instead of the first or second day. The experimenters are now attempting to discover the reason for this. It may have great practical importance as the mortality in cases where treatment was delayed until the third day was less than 5 per cent., compared with 27 per cent. for the first day and 34 per cent. for the second day. L. G.

Rabies Menace Increases; Medical Men Urge Action. Science News Letter, May 29, 1937. A quicker and more positive test for rabies and a less cumbrous method of vaccination must be found, declared the *Journal of the American Medical Association* in its leading editorial of the week (May 22).

Last year's alarming situation in regard to dog-bites and rabies appears even more menacing this year, the medical journal asserts.

More than 100 persons have been bitten by dogs daily in Chicago alone in recent warm days, a 50 per cent. increase over the number of bites during the similar period of 1936.

"Immediate and coordinated action is necessary," the *Journal* states. "Rabies is a disease in which individual efforts are relatively helpless unless aided by the full machinery of social organization.

"The press, public health officials, the police and physicians—in both their individual and their official capacities—should take steps to combat this threatening situation at once if a considerable number of unnecessary deaths is to be avoided.

"In the face of the now existing information as to the frequency and rapid spread of rabies among animals, it seems criminal to postpone action until the disease is identified in human beings.

"Because rabies is primarily a disease of dogs, it seems likely that this campaign will have the whole-hearted support of all the animal humane societies." L. G.

Wars Won With Quinine Instead of Artillery. Science News Letter, June 12, 1937. Medical men can point with pride to their achievements in the field of military medicine. If in the future men are determined to kill each other in wars they can be sure they will meet their death in or because of battle. They will not be killed or kept out of the fray by the diseases which used to take more lives of fighting men than guns or swords.

How preventive medicine has triumphed in this field appears in a report of Sir Aldo Castellani, newly-created Count of Kisymaio, who was Inspector General of the Italian Army and Navy medical services during the Italo-Ethiopian War.

More than 20,000 men in the Italian Army should have died of disease, according to experience of previous colonial wars in tropical countries in which white troops were chiefly employed, Sir Aldo points out. The actual number of deaths from disease was 599, and this included deaths from motor accidents and drowning.

Malaria, expected to be the chief scourge of the white troops, was kept at a minimum, with the number of deaths "infinitely lower than expected" by quinine. Each soldier was given 3 tablets a day and took them, example of the officers being the chief coercive measure.

Dysentery, the "blood flux" which generals used to fear more than the enemy, did not take a single life and affected only 453 men, whereas at least 80,000 to 100,000 might have been expected. The preventive measures against this disease were threefold: 1. Every effort was made to give the officers and men pure drinking water; 2. The men were encouraged to wash or disinfect their hands with lysol, especially before meals, and cooks and others working in the kitchen were required to use this disinfectant; 3. The "grandmotherly precaution" of wearing a flannel abdominal belt was enforced and every soldier wore one to prevent abdominal chilling which might predispose to dysentery. L. G.

Rabies Found to Occur at Any Season of Year. Science News Letter, June 12, 1937. The cry of "Mad Dog!" will soon be heard over the land. Not, that dogs go mad, or suffer from rabies, any oftener during the dog days of mid-summer than at any other season. But from April to September dog bites are more frequent because at this season more dogs are running loose.

Rabies gets its other name of hydrophobia from the fact that at the height of the disease, swallowing is so difficult the afflicted person or dog will refuse even water. The choking, huskiness and shortness of breath are due to spasms of the muscles of swallowing and breathing. In the later stages, the spasms and convulsions may affect the whole body. Death occurs from paralysis of the breathing muscles.

This truly horrible disease is caused by a virus, one of those ultra-microscopic substances which latest scientific discoveries show may be chemicals rather than living organisms. When a rabid dog or other animal bites, he passes the virus to his victim through the saliva. L. G.

Tested Formulas, Deodorants. Joseph Kalish. *Drug & Cos. Ind. 40,* 352 (1937). The author discusses the various kinds of deodorants from the standpoint of the manner in which they operate, that is, by chemical action upon the perspiration or by checking perspiration entirely.

The first group includes zinc oxide, precipitated chalk, boric acid and zinc peroxide, while the second embraces such astringent type substances as aluminum salts, formaldehyde and ferric chloride.

Powders are the least satisfactory of deodorant preparations because of the difficulty in application. They must be of fine texture and be able to cling to the body when applied.

Liquids are most popular and usually are of the astringent type. Aluminum sulfate solutions in concentrations up to 25 per cent. are quite sastisfactory and are preferred to aluminum chloride solutions which liberate hydrochloric acid upon hydrolysis, the latter causing disintegration of clothes with which it comes in contact. Aluminum acetate solutions are also useful. One per cent. of boric acid may be added to maintain a clear solution. Alcohol aids in quick evaporation but may precipitate the salts from solution. Formaldehyde is considered objectionable due to its irritating properties but preparations of methenamine offer the advantages without the disadvantages of the former. Salves and creams are quite satisfactory but must be exceptionally smooth. Sticks are becoming more popular.

The following formulas are appended as typical deodorants and may be changed and altered to meet conditions:

Paste		*Cream*	
Boric acid	15	Glyceryl monostearate	20
Zinc oxide	20	Glycerin	10
Petrolatum	65	Methenamine	5
		Water	65

Paste		*Stick*	
Hydrogenated oil	85	Beeswax	20
Basic aluminum acetate	15	Paraffin	15
		Petrolatum	20
		Mineral oil	35
		Zinc peroxide	10

A. B. N.

Tested Formulas—Brushless Shaves. By Joseph Kalish. *Drug & Cos. Ind. 40,* 496 (1937). An interesting comment on one of the reasons for the popularity of brushless shaving creams is their non-corrosive action on razor blades, as compared to the corrosive and dulling action of an alkaline cream as found in lathering creams. The latter alkalinity may be irritating to sensitive skins also.

Brushless creams, essentially oil-in-water vanishing creams, require 5 to 10 per cent. of glycerin or a suitable substitute to prevent

drying of the cream. Lanolin, spermaceti, cocoa butter, vegetable oils, etc., are used to prevent a drying effect on the skin. One per cent. of cetyl alcohol is valuable as an emollient. The texture of the cream should be such that it will spread readily on a wet face and yet heavy enough to support the hairs. Mineral oil is a frequent ingredient.

The author includes a table showing various basic soap combinations and discusses them from the viewpoint of manipulation, firmness and ease of dilution.

The following formulas are included in the article to indicate the general types which are available. These may be adjusted to suit the demand:

I		IV	
Stearic acid	35.5	Stearic acid	20.0
Sesame oil	4.0	Cetyl alcohol	0.5
Glycerin	5.0	Mineral oil	2.0
KOH	0.9	KOH	1.0
Water	54.6	Glycerin	7.0
		Water	69.5

II		V	
Stearic acid	15.0	Glyceryl monostearate	12.0
Cetyl alcohol	2.0	Mineral oil	2.5
Lanolin	3.0	Spermaceti	4.5
Triethanolamine	0.6	Diethylene glycol ether	7.0
Borax	0.4	Water	74.0
Glycerin	4.8		
Water	74.2		

III		VI	
Glycol stearate	15.	Stearic acid	24.0
Cocoa butter	4.	Oleic acid	2.0
Stearic acid	5.	KOH	0.6
Mineral oil	1.	NaOH	0.2
Glycerin	5.	Glycerin	8.5
Water	70.	Water	64.7

A. B. N.

Lead Poisoning Rates Worst as a Hazard to Workers. Science News Letter, May 15, 1937. Lead poisoning, and not silicosis or any

of the other newly prominent occupational diseases, is the chief hazard to the health of workers in industry, Dr. William D. McNally of Rush Medical College, Chicago, reported to the Midwest Conference on Occupational Diseases in Detroit.

Carbon monoxide and fumes from oxides of nitrogen in dynamite explosions were described as other serious industrial health hazards.

"Wherever dusts are found containing lead, whether it be in mines, smelting, in the manufacture of lead pigments, or in the manufacture of storage battery plates," Dr. McNally said, "poisoning is certain to result."

"There are over 900 occupations causing injurious effect upon the health of the individuals engaged in them," Dr. McNally said.

Silicosis, caused by inhalation of silica-laden dust, predisposes the lining of the bronchial tubes to attacks of bronchitis, he explained. The bronchitis lays a foundation for later-developing pneumonia and tuberculosis.

Preventive measures must include the examination of every new employe, good ventilation, masks, and the use of wet processes wherever feasible. Post-mortem examinations are advocated in all cases of death where the worker had been engaged in a dusty atmosphere, as microscopical and chemical examination of the lungs will definitely prove whether or not the cause in question is one of silicosis.

Carbon monoxide, one of the most important poisons associated with human life and industry, is without doubt the oldest known poison, Dr. McNally said. Wherever gasoline engines are operated, wherever gas heat appliances are used or wherever there is incomplete combustion of any carbonaceous material, this gas is present. The excellent results obtained in the treatment of carbon monoxide by carbon dioxide and oxygen renders all other methods superfluous.

The danger of inhaling oxides of nitrogen was emphasized because of their delayed action. A workman may leave his job complaining of only a bronchial irritation after inhaling the fumes of a dynamite explosion. Several hours later, his lungs become edematous and death may occur within 24 hours.

Danger in the use of solvents such as benzol, carbon tetrachloride, and trichlorethylene, lies not only in industry but in the home as well. Quantities larger than one pint, Dr. McNally warned, should not be sold to the laity. L. G.

Health Authorities Fight New Yellow Fever Threat. Science News Letter, May 1, 1937. Five steps to be taken immediately to fight off the yellow fever menace which now threatens the country were advocated by the Conference of State and Territorial Health Officers with the U .S. Public Health Service.

A national law prohibiting the establishment or maintenance of airports of entry within 20 miles of any community in which the yellow fever mosquito is prevalent is one of the measures recommended to fight the yellow fever menace which threatens because air travel is bringing yellow fever infested regions of South America dangerously near to this country.

In addition to this measure the federal health service was urged to develop detailed procedure for immediately controlling yellow fever in a community and preventing its spread upon discovery of a single case.

Other steps to be taken include eradication of the yellow fever mosquito from communities adjacent to airports and withholding permits for airports of entry from communities in infectible territory unless the adjacent communities institute adequate measures to control the yellow fever mosquito.

Immediate action was urged upon the conference by Surgeon General Thomas Parran. L. G.

Cobra Toxin and Its Therapeutic Use. M. Kirschen. *Ärztl. Praxis 10,* 313 (1936), through *Pharmaz. Zentrh. 78,* 309 (1937). Cobra toxin may be collected from the cobra which is indigenous to Africa, Asia and in particular India. It is a yellowish, syrupy, tasteless, very poisonous liquid that coagulates upon drying. In this form it is quite stable and may be redissolved in water. Chemically and pharmacologically it is related to the class of toxalbumens, although it acts in contrast to these without an incubation time being required. From it have also been isolated a neurotoxin and an enzyme (Phosphodiastase). Its activity is diminished by x-ray and ultra-violet radiation as well as different chemicals (alcohol, chloroform, iodine, silver salts and acids).

The bite of the cobra is scarcely painful and local reactions do not occur. After about an hour there develops an extreme fatigue, occasionally vomitting and paralysis; death results after about 5 hours from respiratory failure.

In animal experimentation cobra toxin was observed to lower the blood pressure and accelerate respiration and in certain doses the relief of cramps and pain was noticed.

Cobra toxin has been recommended in France as of value in the treatment of cancer. According to the experiments of the author, cobra toxin, if the correct dose is found is valuable in temporarily relieving pain and symptoms in cancer. Those suffering from cancer frequently experience gain in appetite and general health, increase in weight, and are rendered partially able to again work. A complete healing and cure of carcinoma was not observed in any case. Most were again incapacitated after 1 to 2 years and finally died. Of course, the author performed all of his experiments on non-operable cases.

L. F. T.

Water Smelling Now a Vocation. Science News Letter, June 5, 1937. Henry Laughlin of Tyrone, Pa., has just been appointed to the position of "water smeller" by one of the chemical companies which makes activated carbon that serves so usefully in taking the odors out of the water supply of over a thousand cities and towns of the land.

Among Mr. Laughlin's accomplishments is the ability to turn on the faucet in a city and simply by smelling the water indicate its palatability. He is also claimed to have an odor "memory" which allows him to identify water from a city once he has smelled of it previously.

Although people seldom realize it, safe and palatable water can no longer be classed along with air as one of those "free" things in life. Processing America's water costs a billion dollars a year.

Water from a river, so often the source for an industrial inland city, has to have mud, and other sediment removed by filtering, settling and precipitation. That it must be freed of harmful germs goes without saying. Moreover, the ultimate users of the water are becoming more vociferous in their demands for the removal of odors and tastes that lower palatability. The days are rapidly diminishing when a city could simply mix chlorine with the water and make it safe, at least, no matter how it looked or tasted.

And that is where America's first "water smeller" comes in. His job is to advise cities and towns on the removal of odors from their water supply.

L. G.

Chemical Cure for Gonorrhea Reported. Science News Letter, June 5, 1937. Possibility of conquering gonorrhea, by a new chemical treatment appears in the report of Drs. John E. Dees and J. A. C. Colston, Johns Hopkins Hospital, Baltimore, to the American Medical Association in Chicago.

Sixteen out of nineteen patients were cured by treatment with sulfanilamide. In all but two of these recovery occurred within less than a week.

The treatment is still in the experimental stage, the Baltimore physicians point out, but they state that their results, together with reduction in hospital expenses for previously used methods of treating this widespread condition have impressed them profoundly. They recommend careful use of sufanilamide in clinics where large numbers of gonorrhea patients can be closely watched so that accurate evaluation of the treatment can be made. L. G.

Theoretical and Practical Investigations on the Process of Percolation. A bound thesis consisting of 169 pages, by Kurt Feinstein, Zurich. This thesis appeared from time to time in several numbers of *Acta Pharm. Helv.,* Volume *11,* during the past year. In its composite form it represents probably the most complete treatise on percolation and its many aspects ever assembled.

The first part of the work consists of an extensive discussion pertaining to the historical development of the process of percolation with the various contributions made both in America and abroad.

The second part of the thesis is a critical study of the effect of varying the many factors involved in percolation. The drug chosen for this series of studies was Cinchona and the variables were: 1. The shape of the percolator; 2. Moistening and swelling the drug; 3. Maceration in the percolator; 4. Draining and rate of percolation. There follows a discussion and correlation of the results obtained.

The third part of the work deals with experiments and discussions pertaining to certain specialized forms of percolation, namely, repercolation, diakolation and evakolation.

Throughout the entire paper sound reasoning and explanations are presented based on the experimental results obtained. The thesis

should not be overlooked by those interested in obtaining a comprehensive understanding of the various factors involved in percolation as a process. L. F. T.

Six Tracts of Virgin Forest Remain in Pennsylvania. Capitol News, 10, No. 10 (1937). The Department of Forests and Waters reports that there are still six tracts of virgin forest remaining in Pennsylvania as follows:

The Snyder-Middlesworth State Forest Monument, five miles west of Troxelville in Snyder County, with 200 acres of virgin hemlock, pine and hardwood.

The Detwiler Run State Forest Monument in Logan Forest, Huntingdon County, with fifty acres of pine, hemlock and an almost impenetrable undergrowth of rhododendron. The conditions here picture the primeval forest conditions of parts of the State.

The Alan Seeger State Forest Monument along Stone Creek, Huntingdon County, with fifteen acres containing hemlock trees 400 years old.

The Joyce Kilmer State Forest Monument on White Mountain, Union County, containing twenty-one acres of virgin timber, mostly hemlock.

Other trees which have escaped the lumberman's axe can be found in the Mount Logan State Forest Monument on Mount Logan in Clinton County, and in the Cook Forest State Park along the Clarion River in Forest, Clearfield and Clarion Counties.

E. MAC L.

"The great adventure is not so much the joy of understanding as the excitement of endless search; and correspondingly, to science the wonder of knowledge is not the richness of its present revelation but the limitless possibilities of its self-fertilizing growth."

—Frederick Barry in "Scientific Habit of Thought."

SOLID EXTRACTS

By Ivor Griffith, Ph. M., Sc. D.

Despite the form in which this information is presented it may be accepted as trustworthy and up-to-date. Original sources are not listed but they may be obtained upon request.

Newspapers and periodicals, the country over, have featured the debut of Daphnia, as presented by Dr. Viehoever, before the Denver meeting of the American Association for the Advancement of Science. This diminutive drug detective has been the object of an arduous and successful study by Dr. Viehover and his research staff and its future as a useful and accurate test annual seems assured.

It fully meets the standards of the anti-vivisectionists, and the S. P. C. A. will more than likely offer no objections.

But to the effervescent newspaper which hailed the Daphnia as the "smallest test creature" ever used in drug evaluation we say—*Bugs!*

For determining the toxicity of antiseptics we have for decades used a much more microscopic creature,—the banal bacterium itself.

Mal-de-mer, really serious sea-sickness, is not as prevalent as most people believe. Somewhere we learnt from the records of the United Fruit Company that out of 30,000 passengers there were 210 severe sea-sickness cases .69%; out of 12,000 crew, 53 severe cases, .41%; in the U. S. Navy there were 44 cases, a rate of .3 per thousand.

Even the passenger record does not strike one as being particularly high.

To one contemplating a sea voyage this may be consoling information, but out of sheer mischief we remind all such that the word nausea is derived from the Greek naus, meaning ship!

Also, let such be reminded that even fish become sea-sick. So do other creatures. Caesar records some difficulty in the pursuit of the enemy in his African Wars, because his "horsemen had beasts of burden which were weary in consequence of recent nausea of the sea." During the World War recently landed horses were reported lying down, in a cold sweat, and groaning. Poultry and song birds, transported by sea, often become sea-sick. Monkeys, accustomed to being aloft in swaying trees, are subject to mal-de-mer. A quantity of fish being conveyed from the Galapagos Islands to the New York Aquarium were reported sea-sick by their scientific expedition. Trained

seals en route from England to America gave signs of sea-sickness.

But, bon voyage, anyhow!

How whole-heartedly we once believed in the utter simplicity of the elements, those building bricks of universe. And how rudely our faith in their fundamentality was shocked when we were told of their double and multiple isotopic lives.

Then consider the vitamins. How blithely we labelled them A, B, C, D, etc, But latterly we learn that vitamin B, for instance, is a complex sextette or more, and the official christeners have started calling the fractions B_1, B_2, B_3—and all the way to *be*wilder.

But this is what we started to write. Some vitamin researchers have discovered in vitamin B a fraction which when fed to rats keeps them from having their hair turn gray.

Whether this may be translated to a similar performance in humans is another story. With most of us it is not so much a matter of worrying over "silver threads among the gold"—but rather a matter of the fear of no hair at all.

Sometime, and elsewhere, we have described the orderly growth of a crystal from a molecular mob in solution, as being a form of "life."

Recently crystals that moved under their own power, giving a curious illusion of being "alive" were demonstrated in motion pictures by Prof. R. W. Wood of the Johns Hopkins University.

The crystals are of a relatively little studied substance, protocatechuic acid. The phenomenon of these moving crystals was first noticed in 1888 by a chemist named Otto Lehman, and then apparently quite lost sight of.

Under certain conditions, the crystals develop in the form of branched rods like the claws of a lobster. The joints straighten out by a progressive motion of the sharp angle of bend, which in one or two seconds runs along the crystal to the tip, the bent rod becoming straight.

Large clusters of these crystals sometimes form, and there is constant movement of the "colony" as the bent rods straighten out and the finger-like tips come together and fuse.

Drinkers of saccharine liqueurs, cordials and summer spirit drinks may have the edge on those who like their barleycorn beverages dry

(*sec*). For if experiments on rats may be safe criteria of human reactions sugar does make alcohol less toxic.

According to Yale researches the intoxicating effect of alcohol on the brain is powerfully counteracted by the amount of sugar in the blood. The concentration of alcohol needed to kill a rat with one-tenth of one per cent. of sugar in its blood is more than 20 per cent. higher than the concentration needed to kill when the blood sugar has been reduced to seven hundredths of one per cent. But if the blood sugar is raised to two-tenths of one per cent., the alcohol needed to kill must be increased by 60 per cent.

There is a limit to the protective action of sugar, however. The experimenters artificially raised the rats' blood sugar to concentrations impossible to attain in any natural manner, but found that these did not increase the protective action.

The two physiologists naively stated: "Our investigation has been made chiefly on rats, but we have evidence that men react similarly."

Bacteria have a queer sense of arithmetic. They multiply by division. Most people have not the slightest conception however of the fact that, despite their arithmetical paradoxy, they are living, eating, drinking entities demanding a diet, that is diversified and well balanced. They are fastidious eaters.

The bacteriological laboratory dietitian has to have both knowledge and imagination in order to feed his billion times ten billion hungry microscopic mouths.

Like humans, bacteria also require vitamins. Yeast, for example, could not be made to grow on synthetic media without the vitamin called bios. More recently, it has been found that a substance called pantothenic acid is active in stimulating the growth of yeast. The diphtheria bacillus requires foodstuff of a vitamin-like character found in meat extract. A substance from molasses acts like a vitamin in the diet of legume nodule bacteria.

Some bacteria can build new tissue from raw material without outside aid, but other bacteria apparently need some ready-prepared foods in their diet. The amino acids, which are the building stones of the protein molecule, are one example of the sort of ready-prepared food required by some bacteria. They are also essential in the diet of man, but while the same amino acids are essential in the diet of all humans, different bacteria have different requirements in this respect also.